LETTER TO THE EDITOR

Use of the Pup as the Statistical Unit in Developmental Neurotoxicity Studies: Overlooked Model or Poor Research Design?

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Fischer et al. (2008) report that BDE-99 (2,2′,4,4′,5-pentabromodiphenyl ether) and methylmercury exposure to mouse pups on postnatal day 10 disrupted spontaneous behavior, reduced habitation, and impaired learning/memory abilities. The authors employed an experimental design developed in their laboratory stating:

“[i]n this neonatal animal model each of the different treatment groups comprise mice from three to four different litters. Randomly selecting animals from at least three different litters will have the same statistical effect and power compared to the use of litter based studies to evaluate developmental neurotoxicity (DNT) in neonatal mice (Eriksson and Viberg, 2005; Eriksson et al., 2005).”

This approach contradicts accepted practice in DNT studies. The experimental unit used for the statistical analysis of data derived from DNT studies is the litter, not the individual pup (EPA, 2000) “[I]n general, data from developmental toxicity studies in rodents are best modeled using nested models. These models account for any intralitter correlation, or the tendency of littermates to respond similarly to one another relative to the other litters in a dose group,” at p. 73.; OECD, 2007 “[T]he statistical unit of measure should be the litter (or dam) and not the pup,” at p. 4.).

Litter effects over as few as three litters are generally large and statistically meaningful, and treating as few as two pups per litter as independent measurements, as done by Fischer et al., can almost triple the nominal 0.05 alpha level (Holson and Pearce, 1992).

By way of support for their claim, Fischer et al. cite a book chapter (Eriksson and Viberg, 2005) and an abstract presented at the 44th Annual Meeting of the Society of Toxicology (SOT) (Eriksson et al., 2005). The book chapter provides a cursory description of the SOT abstract, and no data is provided in either. With respect to the abstract’s conclusion, Holson et al. (2007) said in their review of statistical issues and techniques for DNT testing that “[t]his conclusion is incorrect.” “[t]here are litter effects on spontaneous motor activity,” and “[i]gno ring litter effects in the statistical analysis of DNT studies is simply not an acceptable practice”.

The Swedish research group has published eight papers since 2002 in Toxicological Sciences using the same design. This is troubling, because repeated publications tend to lend credence to this methodology. The impact of these papers, and others published elsewhere by this group using the same design, is not inconsequential. Their conclusions have resulted in the requirement that a formal guideline DNT test be performed for the European Union (ECB, 2002). Further, the results are driving legislation at the state level in the United States (MSL, 2004), and, at the federal level, where they have been used to set draft reference doses (IRIS, 2006a, b).

Though not the primary topic of this letter, we also point out that the eight papers in Toxicological Sciences used a device called the “Rat-O-Matic” (ADEA Elektronik AB, Uppsala, Sweden) to measure the primary end point, for example, motor activity. In all eight papers, the supporting documentation given for the device was a dissertation (Fredriksson, 1994). We were unable to find peer-reviewed literature validating this piece of equipment’s use in rats or mice, nor were we able to find any further information on the device or its manufacturer other than a listing for the company on the World Trade Markets web site.

Toxicological Sciences is not responsible for how its published research is used. However, Toxicological Sciences does have a professional responsibility to ensure that the peer review process used acts as a quality control system (Grainger, 2007). It is unfortunate that these eight papers were peer-reviewed and subsequently published without adequate data supporting the validity of their design or measuring device, especially when the design obviates an accepted norm in developmental neurotoxicology studies.

Disclaimer: The authors are employed by Albemarle Corporation, a global specialty chemical manufacturer whose product line includes decabromodiphenyl ether (Deca). Deca was the subject of one of the eight publications mentioned in this letter, and was reported to cause developmental neurotoxicity using the questioned methodology.

REFERENCES


